STUDIES ON THE PHYSIOLOGY AND MECHANICS OF EGG PRODUCTION IN THE DOMESTICATED FOWL

A Biometrical Investigation of Weight Variations in Eggs and their Component Parts with special reference to their Behaviour in Clutches

by

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I. INTRODUCTION

The increased attention which has been comparatively recently devoted to the investigation of the details of the complex processes of the formation of the avian egg has been mainly due to the fact that it involves the coordinated activities of organs and parts of organs which differ in their morphology, physiology and embryonic origin.

Moreover, the domesticated fowl possesses certain features which make it a most suitable subject for such investigations; most outstanding of these are its availability, the ease of handling and observation, its prolificacy under controllable artificial conditions and finally, the economic value of the fowl's egg has undoubtedly added to the importance of the subject.

Various methods of approach have been employed in the investigations dealing with the formation of the fowl's egg, and literature on many branches of the subject has accumulated considerably.

The biometrical study of variation in egg weight has gained a great deal of importance in recent years owing to market demand for eggs of superior weight. Hays (1944) and others observed that the continued rise in average production of eggs from year to year in the United States has been accompanied by a decline in egg weight, and that there is marked/-

marked variability in the weight of the eggs produced in the pullet year.

Variation in egg weight has therefore been investigated by many workers in relation to such factors as the rate of production, season, date of hatching, age at first egg, body weight and dimensions, etc. These studies have been undertaken with the object of obtaining knowledge required for the planning of breeding programmes combining high standards of both egg weight and rate of production.

From the point of view of the physiology of egg formation, variation in egg weight supplies valuable material for investigation because of the possibility that it might represent different levels of physiological activities in the production of egg-forming materials. One of the most interesting types of variation in this respect is that which occurs in association with the position of the egg in the clutch and the size of the clutch produced.

Atwood and Weakley (1917) found from their studies on the eggs produced by two groups of White Leghorns that the weight of eggs in a clutch declines from the first to the last egg and that the rate of decline decreases as the clutch becomes longer. Jull (1924) found that the first egg of a two-egg clutch in Barred Plymouth Rocks is heavier than the second/-

second. Philpott (1934) obtained similar results from eggs laid by White Leghorns. Funk and Kempster (1934) confirmed these findings by results obtained from substantial numbers of eggs laid in various clutch sizes by White Plymouth Rocks. The results obtained by Raimo (1946) using both White Leghorns and Rhode Island Reds agree with those found by the other workers.

The laying of eggs in clutches, as has been pointed out by Atwood (1929) is due to the fact that the time period between eggs laid in succession is usually over 24 hours. Eggs laid on successive days are therefore produced at a later hour each day, thus resulting in the time of oviposition gradually approaching the onset of darkness. As hens do not lay at a late hour in the day or during the night, sooner or later one day of oviposition is missed and a new clutch is started on the following day. The rate of laying therefore depends on the length of the time interval between successive eggs, the nearer to 24 hours, the longer the clutch.

It was commonly believed that the day of missed oviposition was due to an egg being held in the oviduct until the following morning. Warren and Scott (1935b) found this to be untrue, and that the real cause of missed oviposition results from the retention in its follicle of the ovum which is next in sequence, and the delay of its ovulation until the following morning. It was also found by the same authors that there exists a time relation between oviposition and the following ovulation, the latter/-

latter taking place on average about 30 minutes after the former although it does not depend on it for its occurrence. It is clear that delayed ovulation explains the cause of the inter-clutch pause of one day which is characteristic of regularly laying birds, while a longer pause is evidently due to a gap in the sequence of maturation of ova. The importance of these facts in relation to the position of the egg in the clutch is that they show that the ovum of the first egg of a clutch following a pause of one day has a longer intra-follicular period of time than subsequent ova.

The final stage of egg-formation begins with the liberation of the ovum from its ruptured follicle and its subsequent entry into the oviduct where it collects its coatings of albumen, shell membranes, and shell. Since variation in egg weight is the outcome of many complex factors which are involved in the processes of egg formation, a few questions suggest themselves as being of primary importance.

The present investigation has been concerned with some of these and in particular with an attempt to evaluate the role of the several component parts of the egg in determining its total weight together with an analysis of the variability in weight bound to occur when eggs are produced in sequence. It was hoped also to arrive at conclusions as to the relative contributions/-

contributions to total egg weight of fluctuations in yolk size and differences resulting from the secretory activity of those parts of the oviduct responsible for albumem and shell formation respectively.

The question of the rate of yolk formation and its effect on yolk size has been investigated by Warren and Conrad (1939). It was found that the rate of ovum growth, as shown by measurements and growth curves, is similar in eggs produced by different hens and in different eggs produced by the same hen. Differences in yolk size were accordingly attributed to different lengths of the period of growth; the first ovum of a clutch, on account of the delay in its ovulation, has a longer growth period and is therefore heavier than subsequent ova.

The mode of secretion of the different fractions of the egg white and the rate of secretion of its soluble proteins were studied by Conrad and Scott (1942). The authors quoted found that the soluble proteins are secreted and accumulated in the tubular glands of the magnum at a constant rate during the interval between the passage of eggs through that region of the oviduct, even when that interval included a pause between clutches; the hourly rate was determined at 4.05% of the amount found in a fully formed egg. The secretion of insoluble mucin, on the other hand, was shown to be conditioned by the mechanical stimulation

of/-

of the yolk and there was no evidence of its preliminary accumulation. It was therefore concluded that the accumulation of the soluble proteins during the inter-clutch pause accounted for the first egg of a clutch being heavier than the rest.

The formation of the egg shell was shown by Burmester, Scott and Card (1939) to take place at a constant rate after the fourth hour from the time the egg enters the uterus until the time of oviposition. Berg (1945) confirmed these results when he found that shell thickness depends on the length of time which the egg spends in the uterus and that the difference in the thickness of the shells of the different eggs of a clutch was related to the time interval of those eggs.

Variations in the rate of passage of the egg in the oviduct have been shown by Warren and Scott (1935b) to be mostly due to variations in the time spent by the egg in the uterus. The maximum variation in the time interval spent by the different eggs in the magnum was determined as 15 minutes.

The time interval between the laying of successive eggs in a clutch differs in clutches of different length, and also among eggs of different positions of the same clutch. Atwood (1929), Heywang (1935) and Berg (1945) found that the mean time interval for all the eggs of a clutch decreases with increasing clutch length. It was also found that apart from the/-

the first egg, the last one of a clutch is preceded by the longest time interval.

It appears that the conditions which cause the variation in weight of any of the three main constituents of the egg are, at least to some extent, independent of each other. Any attempt to uncover the causes which underlie the variation in egg weight must therefore be based on some knowledge of the factors which bring about variation in the weight of each egg constituent.

MATERIAL AND METHODS

The eggs utilised in this investigation were laid by twelve pure-bred Brown Leghorn pullets, some particulars about which are given in Table 1.

The birds were selected for the purpose of this study from the flock of the Institute of Animal Genetics, Edinburgh, where the present work was carried out.

One pen was set aside for housing the twelve pullets under controlled conditions of husbandry. Trapnesting was carried out hourly from 9 a.m. till 5 p.m. throughout the period.

Examination of the eggs started on 4th March and was carried on till the laying cycle of each bird terminated.

Out of a total of 1403 eggs laid during the period 1384 were examined. Six soft shells which were found broken in the nest (5 laid by bird No. T651 and 1 by T1256), 2 eggs which were found to have their yolks ruptured and inseparable from the albumen (both laid by T867) and 11 eggs which were accidentally broken, make up the total of the remaining 19 eggs.

No incidence of disease was reported during the whole period of examination of the eggs and none of the eggs laid contained more than a single yolk. Seven double-yolked eggs from other birds were examined.

Eggs/-

Eggs which were laid on week days were examined on the following morning, those laid during the weekend were kept in an electric refrigerator until they were examined on Monday. No other precautions were taken to guard against spontaneous evaporation.

Though it is realised that this variable would effect the successive eggs in clutches to a different degree, it will be seen from the description of the material which follows that it would tend to lessen rather than increase the differences exposed.

The method used in determining the weight of the parts of the eggs was as follows:

The egg was weighed and then broken about the centre in the ordinary way and the contents carefully poured out into a porcelain ladle. One of the chalazae was held with forceps and pulled gently, thus slightly stretching the chalaziferons membrane, and then cut off near the surface of the yolk. After this the ladle was held over a container and a glass rod put across it above the spout in such a position that when the ladle was tilted the albumen was poured off and the yolk was retained in it. In most cases the yolk slipped out of the chalaziferons matter which was dragged away with the albumen which was then discarded. The ladle was next held under the water/-

water tap, the water turned on gently until the yolk became almost submerged when the flow of the water was carefully increased causing the yolk to rotate evenly. The water was poured off in the same way as the albumen and the yolk washed again twice. The last washing water was drained off and the inside of the ladle dried with filter paper by turning it several times, each time drying the water left behind the yolk which was finally turned into a dry pan for weighing.

The shell was washed under slow-running water until free of albumen, drained and placed in an electric oven for drying at 110°C for two hours. Shell weight was determined after the shell had been allowed to cool. The shell membranes were not separated and their weight was included in that of the shell as variations in their weight are too small to justify a separate determination. All weighings were made to .001 grams.

On a few occasions the remaining chalaza with a mass of thick albumen adhering to it remained attached to the yolk after the albumen had been poured off, but it was often possible to free the yolk by manipulation during washing. Only in a small number of eggs was it necessary to dissect off the adhering chalaza; this was done under water, and when the yolk did not look firm enough the operation was not finished off until the yolk had been transferred to the weighing pan.

This/-

This method was decided upon after a period of preliminary trials before the recording of data was begun. The method agrees in principle with that developed by Curtis (1911) and used later by others (Jull 1924, Asmundson 1931, Philpott 1934) in the determination of weight of shell and yolk by direct weighing and that of the albumen by substracting the total weight of the other two from the weight of the whole egg. This procedure is justified by the impracticability of collecting the entire quantity of albumen for accurate direct weighing.

It was found during the preliminary trials that separating the yolks according to the method used by Curtis, of pouring off the albumen and retaining the yolk in one half of the egg shell and then drying the yolk by rolling it on filter paper involved two risks:

a) Yolks which were not firm enough very often burst during the process.

b) The adhering mass of albumen which was sometimes so closely applied to the yolk could easily escape detection and become a substantial source of error.

TABLE 1

Some Particulars of the Birds Used in the Present Investigation

Bird No.	Date Hatched	Date 1st Egg	Age 1st Egg Days	Date Last Egg	No. of Eggs Laid from 4th March	No. of Eggs Examined
T618	26.3.46	24.9.46	182	12.9.47	129	128
T651	"	12.9.46	170	7.8.47	60	55
T862	2.4.46	5.9.46	156	1.10.47	137	136
T867	"	18.11.46	230	19.9.47	98	96
T885	"	20.9.46	171	9.9.47	89	87
T893	"	17.10.46	198	21.10.47	86	85
T1228	23.4.46	7.12.46	228	11.10.47	164	163
T1256	"	2.11.46	193	13.10.47	129	127
T1290	"	24.10.46	184	10.10.47	148	146
T.1312	"	18.10.46	178	3.10.47	142	142
T1347	30.4.46	25.11.46	109	23.9.47	112	110
T1350	"	2.12.46	216	23.9.47	109	109
TOTAL					1403	1384

An idea of the laying intensity of the birds involved in this investigation is shown by the proportion of eggs laid in different clutch sizes to the total number produced (Table 2).

TABLE 2

Number of Eggs Laid in Clutches of Different Sizes and their Percentage of the total Number of Eggs

Clutch Size	No. of Clutches	No. of Eggs	% of Total Eggs
2 egg	225	450	32.1
3 egg	118	354	25.1
4 egg	48	192	13.7
5 egg	14	70	5.0
6 egg	11	66	4.7
7 egg	1	7	0.5
Eggs laid singly		264	18.8
TOTAL		1403	100.0

The data on a small number of clutches is not complete because of accidental breakage of one or more eggs, or because of one of the other reasons already stated. Such clutches were not included/-

included in the analysis of data presented in subsequent tables*. The seven-egg clutch was also excluded on account of its being the only sample of that clutch size.

The length of a pause in production preceding a clutch is of importance. A pause of seven or more days upsets the usual descending order in the weight of successive eggs of a clutch (Funk and Kempster, 1934; Podhradsky, 1941). There were relatively few long pauses immediately preceding the clutches recorded during the period of investigation.

Table 3 shows that 88.4% of the total number of clutches followed one-day pauses. Only five (1.2%) clutches followed pauses of 7 or more days, while the rest of the clutches followed 2-5 day pauses.

TABLE 3/-

* This explains discrepancy between numbers of clutches in Table 2 and those in subsequent tables.

TABLE 3

Number of Clutches following One-Day Pause and their Percentage of the Total Number of Clutches

Clutch Length	Total No. of Clutches	No. of Clutches following one-day Pause	% Clutches following one-day Pause
2	221	197	89.1
3	113	99	87.6
4	45	39	86.7
5	14	12	85.7
6	11	10	90.9
TOTAL	404	357	88.4

Egg production was fairly good in most of the birds, and those whose record was low or poor (Table 1) were mostly ones which stopped laying early or in which laying became irregular. T651 laid regularly until early June when her performance became erratic and only 10 eggs were produced from then until laying stopped ; the last few eggs laid were soft shelled. T885 started laying on 16th March following a long pause; laying was regular until early June when it became irregular until it finally ceased in August. T867 stopped laying in September, not much earlier in the season than other birds, but she/-

she had two 8-day pauses, one in May and the other in August, as well as a number of shorter pauses throughout. T893 laid regularly until 24th May when laying stopped until 4th August; when laying was resumed it was irregular compared with the pre-pause production. T1347 and T1350 laid regularly and well into the moulting season, but a high proportion of their eggs were laid singly.

The mean weight of the eggs laid was fairly high (Table 4), averaging 60.780 grams or over two ounces. With the exception of T1312, the percentage of shell is quite uniform but rather low when compared with other published figures. The percentage of albumen was highest in T651 which naturally had the lowest percentage of yolk, and the percentage of yolk in T618 was highest for the whole group.

TABLE 4

Mean Weight of the Parts of the Egg as Percentage of the Mean Weight of the Eggs

Bird No.	No. of Eggs	Mean Egg Weight Grams	Per Cent of Egg Weight		
			Shell	Albumen	Yolk
T618	125	55.796 = 0.221	9.49	56.54	34.00
T651	55	61.631 = 0.666	9.32	61.52	29.14
T862	136	61.059 = 0.226	9.24	59.26	31.54
T867	96	61.625 = 0.347	9.46	60.24	30.22
T885	87	59.971 = 0.264	9.19	60.63	30.23
T893	85	57.076 = 0.322	9.34	58.79	31.71
T1228	163	58.764 = 0.197	9.51	59.41	31.03
T1256	127	64.846 = 0.251	9.73	60.68	29.71
T1290	146	61.718 = 0.278	9.15	60.95	29.81
T1312	142	61.746 = 0.314	10.32	59.47	30.34
T1347	110	66.155 = 0.341	9.04	60.73	30.19
T1350	109	58.986 = 0.263	9.12	58.85	31.96
Total	1348	60.780 = 0.258	9.42	59.70	30.99

RESULTS

All the eggs laid by the twelve birds were arranged into their appropriate clutch sizes and their mean weights and the mean weights of their constituent parts calculated for the different positions.

A general perusal of Tables 5-9 suggests that in all the sizes of clutch analysed the mean weights of successive eggs and egg parts follow a regular order in relation to their clutch position; their weights, except those of the shell, decrease with advancing position. This apparent regularity in the behaviour of the component parts, which is also noticeable in the mean weights for the various clutch sizes (Table 14), requires individual description before some of their possible interrelations are examined.

1. The Whole Egg (Tables 5-10 & 14)

The descending order in egg weight in relation to advancing clutch position which is exposed in the present study is in general agreement with results obtained by other authors referred to in the introduction, Table 10 shows a decrease in weight from first to second egg in both the two- and the three-egg clutches that is statistically significant, but in clutches of four or more the difference between any two successive/-

successive eggs is non-significant, though the trend is in the same direction.

Although, as already stated, these results generally agree with those reported previously it has not been possible to make a comparison of the statistical significance of the differences.

In order to test whether the cumulative differences in the longer clutches would show significance, those between mean weights of first and last eggs were examined. On this basis the decrease in egg size for the first three clutch groups were significant but in the last two the ratio of the difference to its standard error did not rise above 1.3. It must be remembered however, that the size of the samples in the latter is relatively small (14 and 11 clutches respectively) and therefore this presents no real obstacle to the view that in longer clutches also the tendency is for successive eggs to be smaller even though the decrease between first and last egg seems to lessen consistently with increasing clutch length.

It seems probable that the consistent trend just mentioned may be related to the average size of egg within clutches which is shown in Table 14. Here too egg weight decreases regularly with increasing clutch size though the differences/-

differences do not reach the level of significance. Since longer clutch length may be taken as indicative of greater intensity of production, these results are in agreement with the significant negative correlation between rate of production and egg weight reported by Hays (1930).

2. Albumen and Yolk

Results in Tables 10 and 12 show that the albumen is responsible for a substantial part of the difference between successive eggs in any clutch group. It is also seen that the effects of clutch position on the egg weight and albumen weight are very similar. The significance of the difference in weight between albumens of successive eggs and the value of the significance ratios of these differences follow practically the same order as those of the egg. These points suggest that albumen may be a major factor in the variations in egg weight in relation to the position of the egg in the clutch.

The behaviour of yolk weight in relation to clutch position (Tables 5-9 and 13) is similar to that of the albumen in that it decreases with advancing position. The rate of decrease however, from one to the next is not in the same proportion in all clutch positions between these two egg constituents. In both albumen and yolk the difference between mean/-

mean weights from one clutch position to the next is statistically significant only in the shorter clutches, as in the whole egg. In the three-egg clutch the difference in weight between second and third yolks is significant while the difference between the egg and the albumen for the same clutch positions is non-significant. The mean yolk weight for the whole clutch (Table 14) shows a decline with increasing clutch length. This is in agreement with results obtained by Atwood and Weakley (Jr.) (1917) and by Jull (1924).

3. The Shell

Shell weight in relation to clutch position differs from that of total egg and the other two egg constituents in that it does not follow a descending order with advancing clutch position. Data in Tables 5-9 show that it actually follows an ascending order in the two-, three- and four-egg clutches. In the two-egg clutch and the final pair of the three-egg ones the differences in weight between shells is statistically significant, but in the three- and four-egg clutches the difference between first and second shell is not (Table 11).

In the five-egg clutch, the first and second shells have exactly the same mean weight and in the six-egg clutch also they are practically identical. In these two clutches either first/-

first or second shell is significantly heavier than any subsequent shell except the last.

The last shell in all clutches is the heaviest, so that in the five- and six-egg clutches the first, second and last shells are heavier than the intermediate ones.

These results are similar in many respects to those obtained by Berg (1945) from his studies on shell thickness in relation to clutch position and time interval between eggs in White Leghorns. Since shell thickness is highly correlated with egg weight ($r = 0.778$ according to Asmundson, 1931) there is justification in comparing Berg's results with the present ones.

The time intervals between eggs (Table 15) are similar to those given by Atwood (1929), Heywang (1938) and Berg (1945). There is complete agreement in that the interval preceding the last egg is greater than any other interval (apart from the inter-clutch gap) and that in the longer clutches the intervals of the intermediate eggs are shorter than those at either end. The conclusion drawn by Berg that the last shell is thickest on account of its egg having the longest interval is supported by the results obtained in this study. Berg's findings differ slightly from the present ones in that he found the first egg of the longer clutches thicker than the second,/-

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second, whereas the present results show that the second shell is either equal to the first or insignificantly heavier.

Whereas shell weight in relation to clutch position behaves differently from that of either albumen or yolk the data in Table 14 show that its mean weight for the whole clutch follows the same trend as that of the other two egg components in that it decreases with increasing clutch length.

3. Variability of measurements contributing to the mean weights.

Since the foregoing descriptions are based on mean weights and the significance of differences between them, some indication of the relative population variability in the different groupings of the material would be an aid in assessing their value. This has been measured by means of the coefficient of variation which is appended to all the mean values in Tables 5-9. Within clutch sizes and within egg components the coefficient shows a considerable degree of uniformity. The extreme individual values are 2.7% for a total egg weight in the six-egg clutch and 11.7% for shell weight in the two-egg clutch.

Within these limits variability in the two-egg clutch is lowest for the egg, highest for the shell, and intermediate in degree for yolk and albumen. With increasing clutch length the egg and egg parts become less variable; total egg remains the/-

the least variable and yolk becomes less so than albumen. The variability in shell weight, which in the two-egg clutch is noticeably much higher than in either yolk or albumen, decreases with increasing clutch length until it becomes similar to that of albumen. Variability in relation to position of the egg in the clutch does not seem to follow a regular order, but it is evident that as the clutch becomes longer total eggs and egg parts become less variable in the latter part of the clutch.

In view of the small number of samples of 5 and 6 egg clutches under examination the general tendency for their coefficients of variation to be lower than in shorter clutches, and the absence of any marked irregularities in individual coefficients adds support to the conclusions about consistent trends deduced from the means themselves.

4. Effect of Season on Egg Weight and Variation in Relation to Clutch Position in Different Months

The foregoing results have shown that the weight of the egg and its constituent parts are influenced by their position in the clutch. The possibility of the existence of a seasonal effect on those results made it necessary to examine the position with regard to egg behaviour in different months. The two-egg clutch was selected for this purpose for a number of/-

of reasons. Eggs in it were laid by every one of the birds involved in this investigation in every month of the laying period, and the total number of eggs laid in two-egg clutches represents 32.1% of the whole material (Table 2) and is higher than that for any other group. Other clutch sizes, especially the longest ones were confined to certain months and also to certain birds. Moreover, the two-egg clutch shows the highest level of significance of difference between the weight of eggs in relation to clutch position.

Comparison of the mean monthly weights of first and second eggs and egg parts of the two-egg clutch are given in Table 16* while differences between first and second eggs and the contribution of each egg part in terms of percentage are given in Table 17. The data expose a tendency for the weights of the egg, the albumen and the yolk to increase as the season advances, but differences between their weights in first and second eggs persisted throughout the whole period, increasing slightly towards the latter part. Shell weight shows a corresponding decrease and the contribution of the shell to the difference in weight between first and second egg is always in the opposite direction to those of albumen and yolk. It thus appears that the influence of clutch position on egg weight/-

* Mean weights in Table 16 were calculated directly from ungrouped data show slight difference from mean weight in Table 15, which were calculated from data grouped in a frequency table.

weight is not confined to any period of the season.

5. Relation between Variation in Yolk and Albumen

The figures in Table 16 also demonstrate that the increase in mean yolk weight from the beginning to the end of the season was accompanied by an increase in albumen weight. In spite of some irregularity in the relative order of their mean values this observation would seem to suggest the existence of a certain relation between yolk and albumen weights. Were this the case there is no reason to suppose that it would be limited to two-egg clutches though from the data already described it might be expected that some modification of the relation due to clutch size might occur.

To examine the validity of the assumption, the correlation coefficient between yolk and albumen weights was determined for the entire normal egg output of the twelve birds used for this study. In view of the seasonal trend just demonstrated the material was considered in two Perids (Period 1, March till end of June and Period 2, July till end of laying cycle). If there were any changes in the relation of the constituents with advancing stages of production it was hoped that theirexistence would be indicated under the classification.

The results of the calculations are set out in Table 18*

*A small number of eggs were eliminated on account of their abnormally small size, hence the reason for the discrepancy between the total number of eggs in Tables 1 and 18.

18* and show that in all but one of the twelve birds yolk weight was significantly positively correlated with albumen weight in Period 1. With one exception the significant values of r have a probability of zero correlation of less than 1%, and that for the twelth bird was in the region of 12%.

It is of interest that the production of the latter (T867), was interrupted by pauses throughout the whole season.

In Period 2 the significant correlation between yolk and albumen weight was continued in some birds while in the rest there was no correlation between yolk and albumen weights.

Examination of the individual records of all the birds revealed that those whose yolk and albumen weights were significantly correlated in both Periods (designated Group A for convenience) had normal laying records. The laying records of the other birds (Group B) showed that each one of them exhibited one or more of the following peculiarities:

a) Stopped laying early in the season.

b) Laying was irregular in Period 2.

c) Laid eggs with shell abnormalities.

Bird No. T651 laid only 10 eggs in Period 2, five of which were soft shelled and were found broken in th4 nest. The remaining/-

* See footnote on p. 26

remaining five had shells of an average weight of 4.254 grams against 5.963 grams in Period 1. Probably more soft shelled eggs had been laid and escaped attention.

Bird No. T885 laid regularly during Period 1, but her laying-performance changed in the second period. Only 10 eggs were laid between 1st July and 14 August. One more egg was laid on 9th September; this was accidentally broken and there is no data for its egg constituents. The mean shell weight for Period 2 was 4.962 grams against 5.569 grams for Period 1.

Bird No. T893 laid regularly until late in May when there was a laying pause lasting until early August. Laying was irregular in Period 2 where mean shell weight was 4.465 grams against 5.732 grams in Period 1.

Bird No. T1256 laid fairly regularly until late in the season, but in Period 2 espectially in the latter part there were certain shell peculiarities: it was often thin and semi-transparent with a granular appearance at the broad end. The thick albumen was found on breaking the eggs to be adhering firmly to the shell membrane at the broad end of the egg. Average shell weight was 5.891 grams in Period 2 and 6.446 grams in Period 1. Pronounced shell weight decrease did not start until near the end of Period 2, and the average shell weight for/-

for the seven eggs laid in October was only 4.821 grams.

Egg laying remained normal in Period 2 in all the birds of Group A, and there were no shell defects. The mean decrease in shell weight in Group A was 0.063 grams as against 0.591 grams in Group B.

It therefore appeared as if Group A and B represented two distinct populations, and this was confirmed by the comparison of their mean yolk and albumen weights for both Periods 1 and 2 together.

For the whole season mean yolk weight was 18.830 grams (standard deviation = 1.212), and 18.162 (standard deviation = 3.306) in groups A and B respectively. Mean albumen weight in Group A was 36.056 grams (standard deviation = 3.062) and in Group B 37.032 (standard deviation 3.260).

The differences between mean yolk weight and mean albumen weight of the two groups are statistically significant. The significance ratio for yolk difference is 3.34, and the difference in albumen 4.93.

Mean yolk and albumen weight was determined for Periods 1 and 2 separately in both groups (Table 19). Results given show that both yolk and albumen weights in the two groups have increased in Period 2. Statistical treatment of the results (Table 21) show that the increase in Group A is significant in both yolk and albumen while in Group B the increase is significant in the yolk only.

TABLE 5

Two-Egg Clutch

Mean Weight, Standard Deviation, and Coefficient of Variation of the Egg and the Egg Constituents
(221 Clutches)

Order in Clutch	Mean Weight Grams	Standard Deviation	Coefficient of Variation
1st Egg	62.853 ± 0.270	± 4.019 ± 0.191	6.394 ± 0.304
2nd Egg	59.980 ± 0.245	± 3.642 ± 0.173	6.072 ± 0.289
1st Shell	5.607 ± 0.039	± 0.588 ± 0.028	10.487 ± 0.499
2nd Shell	5.896 ± 0.046	± 0.689 ± 0.033	11.686 ± 0.556
1st Albumen	37.885 ± 0.209	± 3.115 ± 0.148	8.222 ± 0.391
2nd Albumen	35.360 ± 0.187	± 2.783 ± 0.132	7.807 ± 0.371
1st Yolk	19.171 ± 0.089	± 1.328 ± 0.063	6.906 ± 0.328
2nd Yolk	18.743 ± 0.089	± 1.317 ± 0.063	7.029 ± 0.334

TABLE 6

Three-Egg Clutch

Mean Weight, Standard Deviation, and Coefficient of Variation of the Egg and the Egg Constituents (113 Clutches)

Order in Clutch	Mean Weight Grams	Standard Deviation	Coefficient of Variation
1st) Egg	61.942 ± 0.312	± 3.320 ± 0.221	5.36 ± 0.36
2nd) Egg	59.783 ± 0.284	± 3.017 ± 0.201	5.05 ± 0.34
3rd) Egg	59.013 ± 0.320	± 3.397 ± 0.226	5.76 ± 0.38
1st) Shell	5.702 ± 0.042	± 0.449 ± 0.030	7.87 ± 0.52
2nd) Shell	5.758 ± 0.043	± 0.453 ± 0.030	7.87 ± 0.52
3rd) Shell	5.921 ± 0.055	± 0.584 ± 0.039	9.86 ± 0.66
1st) Albumen	37.226 ± 0.271	± 2.882 ± 0.192	7.74 ± 0.515
2nd) Albumen	35.279 ± 0.238	± 2.530 ± 0.168	7.17 ± 0.48
3rd) Albumen	34.898 ± 0.246	± 2.619 ± 0.174	7.50 ± 0.50
1st) Yolk	19.017 ± 0.089	± 0.951 ± 0.063	5.00 ± 0.33
2nd) Yolk	18.762 ± 0.081	± 0.941 ± 0.063	5.02 ± 0.33
3rd) Yolk	18.198 ± 0.101	± 1.075 ± 0.072	5.91 ± 0.39

TABLE 7

Four-Egg Clutch

Mean Weight, Standard Deviation, and Coefficient of Variation of the Egg and the Egg Constituents

(45 Clutches)

Order in Clutch	Mean Weight Grams	Standard Deviation	Coefficient of Variation
1st) Egg	60.456 ± .632	± 4.243 ± .447	7.02 ± .740
2nd) Egg	59.749 ± .512	± 3.434 ± .362	5.75 ± .606
3rd) Egg	58.749 ± .427	± 2.862 ± .302	4.87 ± .513
4th) Egg	58.345 ± .404	± 2.707 ± .285	4.65 ± .490
1st) Shell	5.562 ± .079	± .530 ± .056	9.53 ± 1.005
2nd) Shell	5.633 ± .079	± .530 ± .056	9.53 ± 1.005
3rd) Shell	5.708 ± .084	± .565 ± .060	9.90 ± 1.043
4th) Shell	5.869 ± .064	± .428 ± .045	7.29 ± .728
1st) Albumen	36.305 ± .434	± 2.913 ± .307	8.02 ± .846
2nd) Albumen	35.550 ± .428	± 2.783 ± .293	7.83 ± .826
3rd) Albumen	34.695 ± .312	± 2.082 ± .219	6.00 ± .633
4th) Albumen	34.528 ± .335	± 2.245 ± .237	6.50 ± .695

TABLE 7 (contd)

Order in Clutch	Mean Weight Grams	Standard Deviation	Coefficient of Variation
1st) Yolk	18.547 ± .254	± 1.703 ± .780	9.18 ± .968
2nd)	18.451 ± .184	± 1.233 ± .130	6.68 ± .704
3rd)	18.347 ± .160	± 1.072 ± .113	5.84 ± .616
4th)	17.997 ± .162	± 1.090 ± .115	6.16 ± .649

TABLE 8

Five-Egg Clutch

Mean Weight, Standard Deviation, and Coefficient of Variation of the Egg and the Egg Constituents

(14 Clutches)

Order in Clutch	Mean Weight Grams	Standard Variation	Coefficient of Variation
1st) Egg	59.357 ± 1.120	± 4.189 ± .792	7.06 ± 1.335
2nd) Egg	59.286 ± .877	± 3.281 ± .620	5.53 ± 1.046
3rd) Egg	57.678 ± .812	± 3.040 ± .574	5.27 ± .996
4th) Egg	57.143 ± .923	± 3.454 ± .653	6.04 ± 1.142
5th) Egg	57.571 ± .748	± 2.800 ± .529	4.86 ± .919
1st) Shell	5.579 ± .098	± .367 ± .069	6.57 ± 1.243
2nd) Shell	5.579 ± .118	± .441 ± .083	7.90 ± 1.494
3rd) Shell	5.279 ± .085	± .317 ± .060	6.00 ± 1.350
4th) Shell	5.471 ± .098	± .365 ± .069	6.67 ± 1.274
5th) Shell	5.657 ± .069	± .257 ± .049	4.54 ± .858

TABLE 8 (contd)

Order in Clutch	Mean Weight Grams	Standard Deviation	Coefficient of Variation
1st)	35.536 ± .747	± 2.795 ± .528	7.86 ± 1.486
2nd)	35.179 ± .666	± 2.492 ± .471	7.08 ± 1.339
3rd) Albumen	34.322 ± .567	± 2.120 ± .401	6.18 ± 1.167
4th)	33.643 ± .742	± 2.778 ± .525	8.26 ± 1.560
5th)	34.036 ± .610	± 2.281 ± .431	6.700 ± 1.266
1st)	18.214 ± .380	± 1.422 ± .269	7.81 ± 1.475
2nd)	18.607 ± .320	± 1.196 ± .226	6.43 ± 1.215
3rd) Yolk	18.107 ± .313	± 1.170 ± .221	6.46 ± 1.221
4th)	18.000 ± .200	± .749 ± .142	4.16 ± .786
5th)	17.857 ± .210	± .787 ± .149	4.41 ± .833

TABLE 9

Six-Egg Clutch

Mean Weight, Standard Deviation, and Coefficient of Variation of the Egg and the Egg Constituents
(11 Clutches)

Order in Clutch	Mean Weight Grams	Standard Variation	Coefficient of Variation
1st) Egg	58.795 ± .958	± 3.179 ± .676	5.41 ± 1.240
2nd)	58.886 ± .805	± 2.672 ± .560	4.45 ± .968
3rd)	57.477 ± 1.052	± 3.400 ± .725	5.92 ± 1.262
4th)	57.477 ± .469	± 1.557 ± .332	2.71 ± .578
5th)	57.477 ± .520	± 1.723 ± .367	3.00 ± .638
6th)	57.295 ± .631	± 2.094 ± .446	3.65 ± .778
1st) Shell	5.523 ± .104	± .344 ± .073	6.23 ± 1.326
2nd)	5.568 ± .093	± .307 ± .065	5.51 ± 1.176
3rd)	5.241 ± .098	± .326 ± .070	6.22 ± 1.326
4th)	5.332 ± .068	± .225 ± .048	4.22 ± .900
5th)	5.423 ± .108	± .359 ± .077	6.66 ± 1.420
6th)	5.677 ± .077	± .256 ± .055	4.51 ± .962

TABLE 9 (contd)

Order in Clutch	Mean Weight Grams	Standard Deviation	Coefficient of Variation
1st Albumen	35.069 ± .669	± 2.218 ± .473	6.33 ± 1.348
2nd Albumen	34.795 ± .584	± 1.938 ± .413	5.57 ± 1.171
3rd Albumen	34.386 ± .701	± 2.326 ± .496	6.79 ± 1.448
4th Albumen	34.205 ± .447	± 1.484 ± .316	4.34 ± .925
5th Albumen	33.841 ± .405	± 1.345 ± .287	3.97 ± .847
6th Albumen	33.886 ± .502	± 1.666 ± .355	4.91 ± 1.047
1st Yolk	18.192 ± .351	± 1.163 ± .248	6.40 ± 1.364
2nd Yolk	18.557 ± .273	± .905 ± .193	4.88 ± 1.038
3rd Yolk	17.807 ± .368	± 1.220 ± .260	6.85 ± 1.461
4th Yolk	17.848 ± .197	± .652 ± .139	3.65 ± .780
5th Yolk	17.648 ± .228	± .757 ± .161	4.29 ± .915
6th Yolk	17.602 ± .199	± .661 ± .141	3.75 ± .800

TABLE 10

Significance of Difference Between Means of Weights of Successive Eggs in Different Clutch Sizes

Egg Compared	No. of Clutches	Clutch Size	Diff Betwn Mean. Grams	S.Error of Difference	Diff.÷ S.Error	Significance
1st-2nd	221	2 egg	2.873	0.365	7.9	Significant
1st-2nd	113	3 egg	2.159	0.422	5.1	"
2nd-3rd			0.770	0.428	1.8	Not Significant
1st-2nd	45	4 egg	0.707	0.814	0.9	" "
2nd-3rd			1.000	0.666	1.5	" "
3rd-4th			0.404	0.587	0.7	" "
1st-2nd	14	5 egg	0.071	1.421	0.05	" "
2nd-3rd			1.608	1.196	1.3	" "
3rd-4th			0.535	1.229	0.4	" "
4th-5th			-0.428	1.179	0.04	" "
1st-2nd	11	6 egg	-0.091	1.251	0.07	" "
2nd-3rd			1.409	1.304	1.08	" "
3rd-4th			0.000	1.127	0.00	" "
4th-5th			0.000	0.700	0.00	" "
5th-6th			0.182	0.760	0.2	" "

TABLE 11

Significance of the Difference Between Means of Weights of Shells of Successive Eggs in Different Clutch Sizes.

Shells Compared	No. of Clutches	Clutch Size	Diff Betwn Mean. Grams	S.Error of Difference	Diff.÷ S.Error	Significance
1st-2nd	211	2 egg	-0.289	0.063	4.9	Significant
1st-2nd	113	3 egg	-0.056	0.055	1.0	Not Significant
2nd-3rd			-0.163	0.069	2.3	Significant
1st-2nd			-0.071	0.109	0.7	Not Significant
2nd-3rd	45	4 egg	-0.075	0.114	0.7	" "
3rd-4th			-0.161	0.105	1.5	" "
1st-2nd			0.000	0.153	0.0	" "
2nd-3rd	14	5 egg	+0.300	0.145	2.0	Significant
3rd-4th			-0.192	0.130	1.5	Not Significant
4th-5th			-0.186	0.118	1.6	" "
1st-2nd			-0.045	0.138	0.3	" "
2nd-3rd			+0.327	0.134	2.5	Significant
3rd-4th	11	6 egg	-0.091	0.126	0.7	Not Significant
4th-5th			-0.091	0.126	0.7	" "
5th-6th			-0.254	0.134	1.95	" "

TABLE 12

Significance of Difference Between Mean Weights of Albumens of Successive Eggs in Different Clutch Sizes

Albumen Compared	No. of Clutches	Clutch Size	Diff Betwn Mean. Grams	S.Error of Difference	Diff. ÷ S.Error	Significance
1st-2nd	211	2 egg	2.525	0.281	9.0	Significant
1st-2nd	113	3 egg	1.947	0.361	5.4	"
2nd-3rd			0.381	0.342	1.1	Not Significant
1st-2nd	45	4 egg	0.755	0.600	1.3	" "
2nd-3rd			0.855	0.518	1.6	" "
3rd-4th			0.167	0.456	0.4	" "
1st-2nd	14	5 egg	0.357	1.000	0.4	" "
2nd-3rd			0.857	0.874	1.0	" "
3rd-4th			0.679	0.934	0.7	" "
4th-5th			-0.393	0.960	0.4	" "
1st-2nd	11	6 egg	0.273	0.888	0.3	" "
2nd-3rd			0.409	0.913	0.4	" "
3rd-4th			0.181	0.832	0.2	" "
4th-5th			0.364	0.604	0.6	" "
5th-6th			-0.045	0.464	0.1	" "

TABLE 13

Significance of Difference Between Means of Weights of Yolks of Successive Eggs in Different Clutch Sizes

Yolk Compared	No. of Clutches	Clutch Size	Diff Betwn Mean. Grams	S.Error of Difference	Diff. ÷ S.Error	Significance
1st-2nd	211	2 egg	0.428	0.126	3.4	Significant
1st-2nd	113	3 egg	0.255	0.126	2.0	"
2nd-3rd			0.564	0.134	4.2	"
1st-2nd			0.096	0.313	0.3	Not Significant
2nd-3rd	45	4 egg	0.104	0.243	0.4	" "
3rd-4th			0.350	0.228	1.5	" "
1st-2nd			-0.393	0.496	0.8	" "
2nd-3rd			0.500	0.458	1.1	" "
3rd-4th	14	5 egg	0.107	0.374	0.3	" "
4th-5th			0.143	0.290	0.5	" "
1st-2nd			-0.364	0.444	0.8	" "
2nd-3rd			0.750	0.458	1.6	" "
3rd-4th	11	6 egg	-0.041	0.417	0.1	" "
4th-5th			0.200	0.302	0.7	" "
5th-6th			0.046	0.303	0.1	" "

TABLE 14

Mean Weight of Eggs and Egg Parts of the Whole Clutch in Different Clutch Sizes

Clutch Size	No. of Clutches	Mean Weight in Grams			
		Egg	Shell	Albumen	Yolk
2 egg	221	61.416 ± 0.257	5.751 ± 0.042	36.622 ± 0.198	18.957 ± 0.089
3 egg	113	60.248 ± 0.916	5.794 ± 0.047	35.801 ± 0.252	18.659 ± 0.090
4 egg	45	59.325 ± 0.494	5.693 ± 0.077	35.270 ± 0.377	18.336 ± 0.190
5 egg	14	58.207 ± 0.896	5.513 ± 0.094	34.543 ± 0.666	18.157 ± 0.285
6 egg	11	57.901 ± 0.687	5.460 ± 0.110	34.364 ± 0.662	17.943 ± 0.323

TABLE 15

Average Time in Hours taken by each Egg in the Clutch

Clutch Size	Order of Egg in Clutch						Time Interval*
	1st	2nd	3rd	4th	5th	6th	
2 egg	44.0	28.3					28.3
3 egg	42.6	26.3	27.7				27.0
4 egg	42.0	25.4	26.0	27.2			26.2
5 egg	41.7	26.0	24.8	25.5	26.7		25.75
6 egg	41.7	25.2	24.5	24.9	24.8	26.8	25.24

* Mean Interval between Successive Eggs.

TABLE 16

Mean Monthly Weight of Eggs and Egg Parts of 1st and 2nd Eggs of Two-Egg Clutch

Month	No. of Clutches	Mean Monthly Weight (Grams)							
		1st Egg				2nd Egg			
		Egg	Shell	Albumen	Yolk	Egg	Shell	Albumen	Yolk
Mar.	33	61.830	5.830	37.298	18.703	59.718	6.228	34.958	18.532
Apr.	29	61.312	5.802	37.880	17.631	59.073	6.032	35.929	17.113
May	35	61.686	5.589	37.466	18.631	58.978	5.768	35.217	17.993
June	21	62.416	5.676	37.956	19.784	59.884	5.967	35.504	18.413
July	35	62.885	5.513	37.908	19.465	60.466	5.919	35.591	18.956
Aug.	39	63.522	5.424	37.938	20.161	60.113	5.589	34.833	19.691
Sept.	23	64.910	5.596	38.791	20.523	62.054	5.909	36.038	20.107
Oct.	6	64.790	5.359	38.790	20.641	60.859	5.522	35.242	20.095
Mean	221	62.662	5.615	37.869	19.179	59.994	5.891	35.377	18.727

TABLE 17

Mean Monthly Difference in Weight of Eggs and Egg Parts Between 1st and 2nd Egg of Two-Egg Clutch, and Percentage of Difference Contributed by Each Egg Part

Month	No. of Clutches	Diff. btwn 1st and 2nd Egg (Grams)	Per Cent of the Difference		
			Shell	Albumen	Yolk
Mar.	33	2.112	- 18.8	110.8	8.1
Apr.	29	2.239	- 10.3	87.1	23.1
May	35	2.708	- 6.6	83.0	23.6
June	21	2.532	- 11.5	96.8	14.7
July	35	2.419	- 16.8	95.8	21.0
Aug.	39	3.409	- 4.8	91.2	13.8
Sept.	23	2.856	- 11.0	96.5	14.6
Oct.	6	3.931	- 4.1	90.2	13.9
Mean	221	2.667	- 10.4	93.6	17.0

TABLE 18

Correlation Between Yolk Weight and Albumen Weight During
PERIOD 1 AND PERIOD 2

Bird No.	Period 1		Period 2	
	No. of Eggs	r	No. of Eggs	r
T618	82	0.429 **	46	0.273
T651	48	0.373 **	5	- 0.515
T862	82	0.482 **	54	0.432 **
T867	55	0.231	40	0.410 **
T885	77	0.434 **	10	0.385
T893	58	0.635 **	27	0.145
T1228	87	0.416 **	75	0.612 **
T1256	69	0.309 **	58	0.158
T1290	81	0.391 **	65	0.666 **
T1312	83	0.291 **	58	0.490 **
T1347	66	0.500 **	44	0.352 *
T1350	63	0.280 *	46	0.564 **
TOTAL	851	0.371 **	528	0.415 **
GROUP A	599	0.358 **	428	0.486 **
GROUP B	252	0.404 **	100	0.104

** Probability of zero correlation of less than 1%
* Probability of zero correlation of less than 5%

TABLE 19

Mean Weight and Standard Deviation of Yolks and Albumen of Groups A and B in Periods 1 & 2

Group	Item	No. of Samples	Period	Weight Grams	Standard Deviation	Diff. in Wt. btwn Periods 1 & 2 Grams
A	Yolk	428 599	2 1	19.588 18.290	1.077 1.060	1.298
	Albumen	428 599	2 1	36.970 35.404	3.119 2.830	1.566
B	Yolk	100 252	2 1	19.980 17.910	1.183 1.135	2.070
	Albumen	100 252	2 1	37.396 36.892	2.490 3.466	0.504

TABLE 20

Significance of Difference Between Mean Yolk and Albumen Weights in Periods 1 and 2

Group	Item	Diff. in Wt. btwn Periods 1 & 2 Grams	S. Error of Difference	Difference / S. Error	Significance
A	Yolk	1.298	0.07	18.5	Significant
	Albumen	1.566	0.197	8.0	"
B	Yolk	2.070	0.141	14.3	"
	Albumen	0.504	0.810	0.62	Not Significant

DOUBLE YOLKED EGGS

As reference is made to double yolked eggs in the discussion which follows, a brief description of seven of these abnormalities is appended hereon. Their weights and that of their component parts are listed in Table 21.

It was mentioned earlier these eggs were laid by birds outside the group which provided the main material for this study. Examination of their records showed that most of them had laid more than one double yolked egg and that they were laid in various positions in clutches of different sizes. In this respect, those in the present series, each of which represents a separate hen, are fairly representative in that they are the first egg of a two- and four-egg clutch, the last egg of a 4, 5 and 6-egg clutch and the second last egg of 7 and 8-egg clutches.

As they came from pens in which no record was kept of the hour at which eggs were laid it was not possible to determine whether the production of these double yolked eggs bore a relation to the time interval before or after they were laid, but Conrad and Warren (1940) found in their material that these periods did not differ significantly from those obtained for single yolked eggs.

TABLE 21

Double-Yolked Eggs

Bird No.	Date Laid	Weight in Grams				
		Egg	Shell	Albumen	Yolk	
T449	6.3.47	79.971	6.756	41.067	....	32.148
T1619	17.3.47	81.035	6.460	41.515	16.520 16.640	33.160
S154	17.3.47	75.414	6.282	38.366	15.188 15.578	30.766
T1842	4.4.47	69.182	5.932	39.029	12.008 12.213	24.221
T650	12.4.47	82.472	6.280	45.552	14.145 15.495	29.640
T902	16.4.47	92.603	7.576	51.567	16.667 16.793	33.460
T594	13.5.47	73.704	6.188	37.220	15.165 15.131	30.296

DISCUSSION

Results of the present investigation show that both the length of the clutch and the position of the egg in it are associated with differences in egg weight and the weight of egg parts. Data in Table 14 show a gradual decrease in all these as the clutch becomes longer. Within each clutch length (Tables 5-9) it is seen that egg weight and the weight of each one of its parts are influenced by their position. The weights of the whole egg, the albumen and the yolk show a generally descending order as their position in the clutch advances while the general trend for shell takes the opposite direction.

It would therefore seem that the factors which operate to bring about these differences are not directly the same for each part of the egg.

A discussion of the physiological significance of the differences in weight which is associated with the length of the clutch and the position of the egg in it is given below for each one of the egg parts, taking them in their order in the process of egg formation.

1. The Yolk

The mean yolk weight in clutches of different length (Table/-

(Table 14) shows a gradual decrease from shorter clutches to longer ones, thus indicating that an increased rate of ovulation is concurrent with a decrease in the weight of the ova produced.

The question now arises whether the smaller weight of yolks in long clutches is due to a slower rate of ovum growth brought about by the presence of a bigger number of ova undergoing different stages of maturation. Since the rate of ovum growth is constant either for ova produced by different birds or for different ova produced by the same bird (Warren and Conrad, 1939) the results obtained in the present investigation would appear to confirm their conclusion that increased clutch length results in the shortening of the growth period of the ovum. In other words, increased ovarian activity is associated with early ovulation.

This conclusion is supported by the experimental evidence of the possibility of shortening the period of ovum growth by the induction of premature ovulation.

Fraps, Olsen and Neher (1942) found that exogenous luteinizing substances could cause the induction of ovulation earlier than its expected time up to a maximum of at least 17 hours. It was also found that, apart from the relative potency of the substances used, the degree of 'prematurity' of the ovum determined the effectiveness of the ovulatory agent. A greater degree of prematurity required a higher level/-

level of dosage until a certain limit was reached beyond which increased dosage was ineffective in inducing ovulation. In the light of these results it appears that the ovum becomes ovulable over a fairly long period of its final growth stage, and that the actual time of its ovulation depends on the L.H. level. It would therefore seem justifiable to conclude that the lengthening of the clutch, which indicates increased follicular stimulation, is associated with increase in the level of the ovulatory agent. Furthermore, this conclusion agrees with the stress which has been laid in recent years on the importance of the maintenance of a balance between the levels of the follicle stimulating and the luteinizing hormones for the normal functioning of reproductive activities. Witschi (1940) found by the assay of the gonadotropic potencies of the anterior pituitaries in 14 mammalian species that the FSH/LH quotient is related to species specificity, and so within a species there may be a limited range of balance between the two. Follicular stimulation accompanied by the failure of ovulation and subsequent regression of the mature follicles and the cessation of laying following treatment with anterior pituitary extract has been reported by a number of investigators. Phillips (1943) suggested that this may be due to the upsetting of the hormonal balance of the treated birds. Fraps, Riley and Olsen (1942) found that the response to ovulatory/-

ovulatory substances in hens which were pretreated with follicle stimulating hormones was quicker and more uniform than the response of normally laying non-pretreated hens. The results obtained by Nalbandov and Card (1946) point to the importance of the FSH/LH balance for the continuance of normal ovulation.

It seems fairly obvious from experimental evidence that increased follicular stimulation must be synchronized with increased level of the ovulating agent if egg laying is to go on normally. This increased LH level in its turn results in the curtailment of the growth period of the ovum.

In the present study it was found that yolk weight declines as the position of the egg in the clutch advances. It has already been mentioned that the ovum of the first egg in the clutch has a longer intra-follicular period of time than subsequent ova owing to the delay of its ovulation. Warren and Conrad (1939) found no evidence of retardation in the initiation of the period of growth or of slowing down of the growth rate of the ovum whose ovulation is to be delayed and which is to become the yolk of the first egg in the clutch. It was therefore concluded that this ovum is heavier than subsequent ova of the same clutch because of its longer growth period caused by its retention in its follicle for an additional period of time. Reference to Table 13 shows that while this explanation seems adequate in the case of the two- and the three-egg/-

egg clutches, where the first yolk is significantly heavier than the second, further explanation is required to show why this does not hold in the case of the longer clutches. It may be however, that the phenomenon established in the smaller clutches is merely being obscured by the smallness of the samples in the longer ones. The fact that mean differences in other measurements tends to lessen with increasing clutch length makes it logical that in this particular case also they should become less evident. It is clear that this general trend is based on some fundamental mechanism, possibly the amount of follicle stimulating hormone present in relation to the number of yolks undergoing simultaneous development, but the solution of the problem is outside the scope of the present investigation.

It must also be realised that the sequence of the initiation of the growth period of the successive ova cannot take place with exact regularity; proof of such irregularity is represented in its extreme in what must be the simultaneous initiation of the growth period of two ova culminating in the production of a double yolked egg with two equal sized yolks.

Furthermore, it is not improbable that irregularities in the weight of successive ova may be caused by fluctuations in the level of the ovulating factor during the course of the clutch.

It/)

It would seem that the assay of both the follicle stimulating and the luteinizing factors of the anterior pituitary under various conditions of egg-laying intensity might yield results of considerable interest, in relation to the trends and variations on yolk weights just considered.

2. The Albumen

The explanation put forward by Conrad and Scott (1942) that the accumulation of soluble proteins during the inter-clutch pause is responsible for the first egg of a clutch being heavier than subsequent ones has already been referred to. This explanation, as far as the writer is aware, stands as the only one in the literature surveyed which is based on actual measurements by quantitative chemical methods of the rate of secretion of soluble proteins in the oviduct.

Reference to Table 15 shows that in each clutch length the period preceding the first egg is approximately 16 hours longer than that preceding the second egg of the same clutch. Since this difference corresponds roughly to the delay in ovulation of the ovum of the first egg this result agrees with the findings of Warren and Scott (1935b). The mean time intervals given in Table 15 have also been found to agree with those given by authors already quoted. It therefore appears that in any clutch size the difference in length of time interval of/-

of first and second eggs is about the same. In view of this fact, and if the accumulation of soluble proteins were the sole cuase underlying the difference in weight between the albumens of first and second egg, that difference ought to exist in all clutch sizes, and be fairly constant in degree. Results presented in Table 12 show, as far as the findings of the present investigation are concerned, that this is not the case and that in fact it decreases rapidly with increasing clutch length. The difference between the weight of egg white of first and second egg is significant in the two-egg clutch, also significant, but to a lesser degree in the three-egg clutch, and non-significant in longer clutches.

Even allowing for the descending order of weights of second eggs in clutches of successive sizes and calculating on a basis of 6% of albumen added to all first eggs by the delay in ovulation, the differences shown in the two- and six-egg clutches would be expected to be about 2.12 grams and 2.08 grams respectively as opposed to the observed ones of 2.525 grams and 0.274 grams. Therefore, whatever the fate of the excess of soluble protein accumulated during the inter-clutch interval some basis of variation is necessary to explain the behaviour of albumen weights within the egg.

In view of the different physiological conditions under which/-

which the soluble protein and mucin are secreted, the possibility that this second component of egg white is responsible for the trends observed must also be considered. While Conrad and Scott (1942) concluded that its secretion was dependent on stimulation provided by the presence of the ovum in the oviduct, they did not consider the possibility that the size of the yolk might affect the degree of stimulation induced.

The correlations computed in this study however, can only be regarded as supporting such a view. Those birds which laid normally and regularly throughout the months under review showed a consistent positive correlation between yolk and albumen for both the periods into which the season was divided.

In this connection it is interesting to speculate that if the stimulation is of a mechanical nature then the amount of soluble protein present may also play a part in influencing the degree of stimulation of mucin secretion. If this were so, then the combined bulk of the accumulated soluble protein and (largest) first yolk of a clutch would be expected to result in a difference in total egg albumen of a higher order between the first two eggs than between any other successive pairs in the clutch. While this is certainly the case in the three-egg clutch it cannot be demonstrated in longer ones where/-

where the sample numbers are much smaller.

It was only in birds which developed obvious abnormalities in the production cycle or in shell characteristics that the relationship between yolk and albumen broke down, and then only when these atypical phenomena had actually appeared. While it is not unexpected that the relationship would be upset under these circumstances which reflect physiological disturbances, it is interesting to note that some similarity in the type of breakdown can be traced in the individual histories of these birds given on p.27 et seq. In all of them shell weight dropped to a markedly greater extent than in the more normal group between Periods 1 and 2. In view of the conclusion arrived at on shell variations this is suggestive of a more rapid expulsion of the eggs from the uterus. In that case since Scott, Hughes and Warren (1937) have shown that 50.7% of the albumen accumulates in the egg during its sojourn in this part of the oviduct it would be expected that this would be reflected in the seasonal change in albumen and cause a lessening of the expected increase of that component in Period 2. This is indeed what happens and is clearly demonstrated in Table 22. The increase in mean yolk weight between the two Periods is of a similar order and highly significant in both Groups A and B, but the corresponding increase in albumen in Group B is not significant and is only about a third of the

increase/-

increase established in Group A.

It may be claimed that two independent sources of variation in albumen weight are exposed in the present study: (1) that due to differences in the amount of mechanical stimulation provided by the size of the yolk and possibly the associated soluble protein, and (2) the time during which the egg is in the uterus.

The conclusion that the size of the mechanical stimulant is a factor in determining the amount of albumen is supported by the data from the double yolked eggs (Table 21) which were examined in the present investigation.

They show that each one of them contains more albumen than the amount expected in an egg with a single yolk, equal in size to either of the double yolks. It also appears that the quantity of white is less than the total expected in two separate eggs each with one yolk similar in size to one of the double yolks. Some of those eggs, the ones which were laid during the course of a clutch or at the end of one, are of particular interest in that the increased quantity cannot be due to extra accumulation of soluble protein considering that the interval preceding them could not have been of any exceptional length. There seems to be little doubt that the increased amount is due to the added mechanical stimulus of the extra yolk.

This/-

This view is further substantiated by the data given by Romanoff and Hutt (1945) on 10 double eggs laid by the same hen. The eggs were of the type in which the enclosing egg contained one complete egg and yolk held closely together by one mass of firm white and with common shell membrane. This was taken as evidence that the enclosed egg on its backward journey up the oviduct must have met the oncoming yolk about the anterior end of magnum. The record shows that the association of these eggs with pauses was not particularly different from that of single eggs laid by the same bird except the double eggs always followed a day on which no eggs were laid; that day is, of course, the one on which the enclosed egg would have been laid. The enclosing egg contained on average an abnormally large quantity of white (78.66 grams) more than one and a half times as much as that of the enclosed egg (50.67 grams) which in its turn contained more than that of average single eggs laid by the same bird (46.25 grams). There seems to be no explanation for the huge increase in the albumen content of the enclosing egg other than that given for the double yolked egg; the difference in the proportion of the increase in the two cases is seemingly due to the difference in the bulk of the extra stimulus caused by the presence of one whole egg in one case and of one extra yolk in the other.

Together/-

Together the data furnished by the yolk albumen correlations, the double yolked eggs, and double eggs, supply reasonable evidence that substantial increases or decreases in the egg albumen are a function of secretory activities which show a marked response to the size of a mechanical stimulus. Whereas the soluble proteins are secreted independently of the size or even the presence of a mechanical stimulus, the secretion of mucin is consequent upon both.

The value of the correlation coefficients (Table 18) also support the above conclusions. If the yolk size influenced the whole of the albumen as opposed to the mucin component alone the correlations between yolk weight and albumen weight would have been expected to show somewhat higher values.

3. <u>The Shell</u>

It has been shown in Tables 5-9 that whereas the mean weights of yolk and albumen show a more or less descending order with advancing clutch position, the shell weight follows, with some modification, the reverse order.

One remarkable fact, however, is the obvious relation between shell weight and the time interval preceding the egg. This relation is consistent throughout all clutches of different length, and the results are similar to those obtained by Berg (1945) for shell thickness.

The/-

The time interval preceding the first egg of the clutch differs from that of subsequent eggs in that it includes the additional period of delay in ovulation of its ovum; since this period was found to vary up to 3 or 4 hours (Warren and Scott, 1935b) it is not possible to determine directly the length of time spent by the first egg in the oviduct.

When the time intervals and shell weights of the five clutch groups are arranged in descending rank order the association between them is strikingly clear. This has been done in Table 22 where the descending rank order of yolk and albumen weights are included for comparison.

TABLE 22

Descending Order in Time Interval and the Corresponding Weight of Egg Components under Different Clutches

Clutch Length	Descending Order			
	Time Interval	Shell Wt.	Albumen Wt.	Yolk Wt.
2 egg		2.1	1.2	1.2
3 egg	3.2	3.2.1	1.2.3	1.2.3
4 egg	4.3.2	4.3.2.1	1.2.3.4	1.2.3.4
5 egg	5.2.4.3	5.1.2.4.3	1.2.3.4.5	1.2.3.4.5
6 egg	6.2.4.5.3	6.2.1.5.4.3	1.2.3.4.6.5	2.1.4.3.5.6

The/-

The last shell in any clutch is the heaviest one, thus agreeing with time interval preceding the last egg being longer than that preceding all others.

There is an ascending order of shell weight in the two-, three- and four-egg clutches, but in the five- and six-egg clutches the second and the last eggs are the heaviest while the shells in positions between these are lighter. This corresponds almost exactly with the time interval order if the presence of the first shell is ignored. By inference from these results the latter would be presumed to spend the shortest time in the uterus in each of the first 3 clutch groups, and to have been preceded by a time interval of intermediate length in the two longer ones.

A discrepancy might appear to be present between this suggestion that first eggs are more rapidly expelled and the observation that they also have the greatest amounts of albumen in the shorter clutches. Though it was deduced earlier that the latter is decreased by a shorter time interval the added affect of the soluble proteins accumulated during the inter-clutch gap might be sufficient to counteract this to the extent observed. Consideration of the complicated situation which this represents however, and the presumption that the position can be only slightly less intricate in successive/-

successive eggs makes it clear why the trend of the smaller differences obtained in longer clutches are less easy to trace.

The fact that shell weight follows a descending order with advancing season as opposed to its behaviour within clutches opens up the question of whether the time spent by the egg in the uterus decreases as the production cycle proceeds.

While Burrows and Byerly (1942) have given indications of the hormonal nature of the control of oviposition by inducing it prematurely by means of posterior pituitary perhaps the most significant contribution in this respect is that of Rothschild and Fraps (1944) who showed that the newly ruptured ovarian follicle, and to a lesser extent the maturing follicle, seem to control the time of oviposition.

This suggests a link between the basal causes of variation in shell weight (through time of oviposition) with those determining yolk size. This possibility is of major importance because though mean weight for clutches varies in the same way in the two components, their behaviour, both in different seasons and within clutch groups, is heterogeneous. If this supposed connection concerned the latter, larger yolks would be expected to be associated with early expulsion of the egg and so raise the possibility of their association with deficient/-

deficient shells. It is noteworthy that the increase in yolk size in Period 2 in Group B when the shells were deficient is relatively greater than that obtained in the normal group.

* In T893, one of the birds in which the drop in shell weight was marked between Periods 1 and 2 the correlation between yolk and shell weights was computed for the whole season and was found to be negative and significant being 0.649 ± 0.063.

SUMMARY and CONCLUSIONS

1. The investigation was designed to gain information on the role of the three main components of the egg (yolk, albumen and shell) in determining its total weight., with special reference to the variability known to occur when eggs are produced in sequence.

2. The data used consisted of weight measurements made on the whole egg yield of twelve purebred Brown Leghorn pullets from 4th March 1947 to the end of their respective laying cycles.

3. Clutches examined varied in content from 2-6 eggs; the first two classes contained more than half the total eggs and the last three slightly under a quarter.

4. The overall mean egg weight was 60.78 grams ± 0.258 and the respective corresponding percentages of yolk, albumen and shell, 31.0, 59.7 and 9.4%.

5. The trend for whole egg weight is downward with successive position in the clutch and increasing clutch length; the differences become progressively smaller; that between the first and second egg of the first two clutches is disproportionately large. Egg weight increases as the season under review advances.

6. Yolk and albumen weights showed the same trends to varying degrees.

7./

7. In shells, mean weight fell with increasing clutch size but in the other two groupings (clutch position and season) reverse the order found in the first two components, except that in the 5 and 6-egg clutches, the last, first and second eggs were heaviest.

8. Correlations between yolk and albumen weight were significant and positive for the first part of the season under review, but in four birds this relation broke down in the second part. Compared with those which behaved consistently, the eggs from this group showed a slightly larger increase in yolk weight in the second seasonal period, a noticeable lack of increase in albumen and a marked decrease in shell weight.

9. The data on yolk conforms with the views that delayed ovulation increases the weight of the first egg of a clutch and that changes in size may be hormonally controlled.

10. It was concluded that two major factors may affect albumen variations (a) the changing size of a mechanical stimulus consisting of the yolk plus the soluble proteins secreted between the passage of one egg and another, and (b) the time the egg remains in the uterus.

11. Shell variations could be related to the time interval between/-

between ovipositions and so to the length of sojourn in the uterus.

12. A final inference is that though sources of stimulation such as hormones, might be expected to increase egg weight through an effect on time of ovulation and the secretion rate of soluble proteins, a possible negative relation between yolk weight and time of oviposition might result in a limiting effect on albumen and shell secretion. The question of the actual relations obtaining between ovulation and oviposition therefore, seems to be vital to any attempts to increase egg size by artificial means.

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